Table 2. Reagents affecting histidine phosphatase activities.

Reagent	Concentration	Activity (%)
Phosphate	10 mM	0
Okadaic acid	10 µM	108
Microcystin	5 nM	110
Tautomycin	0.5 nM	90
Inhibitor protein I_1	1 nM	120
Molybdate	10 µM	71
Vanadate	10 µM	62
Fluoride	5 mM	105
EDTA	5 mM	108
EGTA	5 mM	91
Mg^{2+}	1 mM	142
Mg^{2+}	10 mM	226
Ca^{2+}	1 mM	165
Ca^{2+}	10 mM	240

100 % = xxx nmol / min x mg^{-1}

(C) CHARACTERIZATION OF THE HISTIDINE PROTEIN PHOSPHATASE

The purified protein fraction contained a defined band with an apparent molecular weight of 14.000 according to analysis by SDS gel electrophoresis (Figure 4). Mass analysis identified a molecular weight of the protein of 13.768 (Figure 5). The histidine protein phosphatase is N-terminally blocked by an acetyl group. Sequence information is therefore not accessible by Edman degradation and for protein characterization a proteolytic cleavage is required.

(D) PROTEIN ANALYTICAL DETERMINATION

The active fraction (Fig. 4) underwent enzymatic cleavage to determine the amino acid sequence, and the resulting peptide fragments were sequenced by Edman degradation and mass spectrometry applying standard techniques as described in the literature (Kellner R, Lottspeich F, Meyer HE (1999) Microcharacterization of Proteins, Wiley-VCH).

Enzymatic fragmentation

The gel band was cut out using a scalpel and transferred into an Eppendorf tube. The enzymatic fragmentation took place after addition of trypsin as protease (1 µg of trypsin, 100 µl of 0.5 M ammonium bicarbonate, 37°C, 12 h). The resulting peptide fragments were extracted (50% trifluoroacetic acid, 50% acetonitrile). The extract was concentrated in a vacuum centrifuge.

Chromatographic separation of the peptide fragments

The peptide fragments were dissolved in eluent A (0.1% trifluoroacetic acid in water) and applied for separation by reversed phase chromatography (eluent B: 20% 0.1% trifluoroacetic acid in water, 80% acetonitrile). After fractionation based on UV determination at 214 nm (see Figure 6), the separated peptide fragments were in dissolved form and were determined by Edman sequencing and mass spectrometry.

Edman sequencing and mass spectrometric determinations

The liquid fractions after the chromatographic separation were 90% employed for Edman sequencing (standard conditions, apparatus: model 494, PE-Applied Biosystems, Weiterstadt). The remaining part of the fraction was employed for a mass analysis (MALDI-MS), (apparatus: Voyager STR, Perseptive Biosystems, Wiesbaden).

Identified peptide sequences

The following peptide sequences were determined (rabbit):

AAAGLAQIPD VDIDSDGVFK

YVLIR

VHAAPPSEAPGGESK

DIVR

WAEYHADIYDK

VSGELQK

ISHQSQDR

KIHVYGYSMGYGR

YPDYEVTWADDGY

These peptides lead to the enzyme peptide from rabbit (SEQ. No. 6):

AAAGLAQIPDVDIDSDGVFKYVLIRVHAAPPSEAPGGESKDIVRGYKWAEYHADIYDKVSGELQK
KGHDCECLGGGRISHQSQDRKIHVYGYSMGYGRAQHSVSTEKIRAKYPDYEVTWADDGY

(E) Nucleotide Sequencing

A rabbit DNA library was screened with primers selected from the achieved amino acid sequence for the histidine protein phosphatasenucleotide sequence. Cloning and sequencing identified the nucleotide sequence given in Figure 7a.

The 327 bases translate for the histidine protein phosphataseprotein sequence beginning at position 11 to 119 (Figure 7b).

Data base analysis

A database search was carried out using the BLAST algorhythm. Homologous proteins could be identified from C.elegans, Drosophila melanogaster, Drosophila pseudoobscura in protein databases.

In nucleotide databases ESTs were identified in human, rat and mouse.

A human homologue has not not been published yet. According to these EST assemblies human histidine protein phosphatase protein was found to have the following sequence (SEQ. No 2 without a methionine residue):

AVADLALIPDVDIDSDGVFKYVLIRVHSAPRSGAPAAESKEIVRGYKWAEYHADIYDKVSGDMQK
QGCDCECLGGGRISHQSQDKKIHVYGYSMAYGPAQHAISTEKIKAKYPDYEVTWANDGY

A rat homologue has not been published yet. According to these EST assemblies rat histidine protein phosphatase protein was found to have the following sequence (SEQ. No. 7):

NGLNTTRGKGSSPLGKDHQELELLTPYPAVKFSVGPTRATRAYPEATLPTSADIYDKVSGELQKN
GYDCECLGGGRISHQSQDRKIHVYGYSMGYGRAQHSVSTEKIKAKYPDYEVTWADDGY

A mouse homologue has not been published yet. According to these EST assemblies mouse histidine protein phosphatase protein was found to have the following sequence (SEQ. No. 8):

MAADLGQIPDVDIDSDGVFKYVLIRVHLAEPSGDPAKECKEIVRGYKWAEYHADIYDKVSGELQR
NGYDCECLGGGRISHQSQDRKIHVYGYSMGYGRAQHSVSTEKIKAKYPDYEVTWADDGY

Table 3: Sequence homology for the protein histidine phosphatase from various species given in % identity.

	1	2	3	4	5	6	7
1 human	100	84.0	65.3	64.3	68.2	38.0	40.3
2 rabbit		100	72.4	71.4	71.0	36.0	42.0
3 mouse			100	67.3	52.7	32.7	33.3
4 rat				100	51.6	30.8	29.5
5 zebra fish					100	33.7	42.1
6 c.elegans						100	39.7
7 drosophila							100